Freeze Survival Assay Protocol

Adapted From "An Improved 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction assay for evaluating the viability of *Escherichia coli* cells." (Wang et al., 2010)

Materials Needed:

15 mL conical tubes or glass test tubes for growing liquid cultures

Appropriate antibiotic stock

LB Broth

Glycerol

LBG

MTS

1 flat-bottom 96-well plate

Slow-freeze vessels

Two freezers

A plate reader

A centrifuge

To make LBG:

For every 10 mL of LB, add 158 µL of glycerol.

Protocol:

1. Prepare 5mL LB liquid cultures (supplemented with appropriate antibiotics and inducers) from stock or a colony from the transformation plate of the desired strains. Allow the cultures to grow for 18-24 hours at 37 degrees Celsius in a shaking incubator.

2. Measure the OD_{600} of each liquid culture and dilute to an OD of 0.1 in LBG.

3. Place 100 µL of each diluted culture into a 1.5 mL Eppendorf tube. Add 20 µL of MTS solution. Mix well.

4. Incubate cultures at 37 degrees Celsius for 20 minutes, leaving the caps open as they incubate.

5. After the incubation period, centrifuge cultures at max speed for 1 minute.

6. Put cultures on ice. Place 100 µL of each culture into one well of a 96-well plate until all cultures are plated.

7. Place the 96-well plate in a plate reader and measure the OD450 of all cultures.

8. Aliquot two sets of 500 µL of the original, undiluted, unreacted cultures into two 1.5 mL Eppendorf tubes. Place one tube of each culture into one of two slow-freezing vessels. Place one slow-freeze vessel into a freezer set at -20 degrees Celsius and one slow-freeze vessel into a freezer set at -80 degrees Celsius.

9. 24 hours later, thaw each set of cultures and centrifuge them at max speed for 2 minutes. Discard supernatant and resuspend pellets in 500 µL of fresh LBG to eliminate any residual enzyme from lysed cells.

10. Repeat the protocol above for both the -20 degree set of cultures and the -80 degree set of cultures. Compare the results.